XXII Congreso Latinoamericano de Bioquímica Clínica

Punta del Este – Septiembre 20, 2017

(Auditórium Principal – 14:10 – 15:40)

Resistance to carbapenems: mechanisms and detection methods

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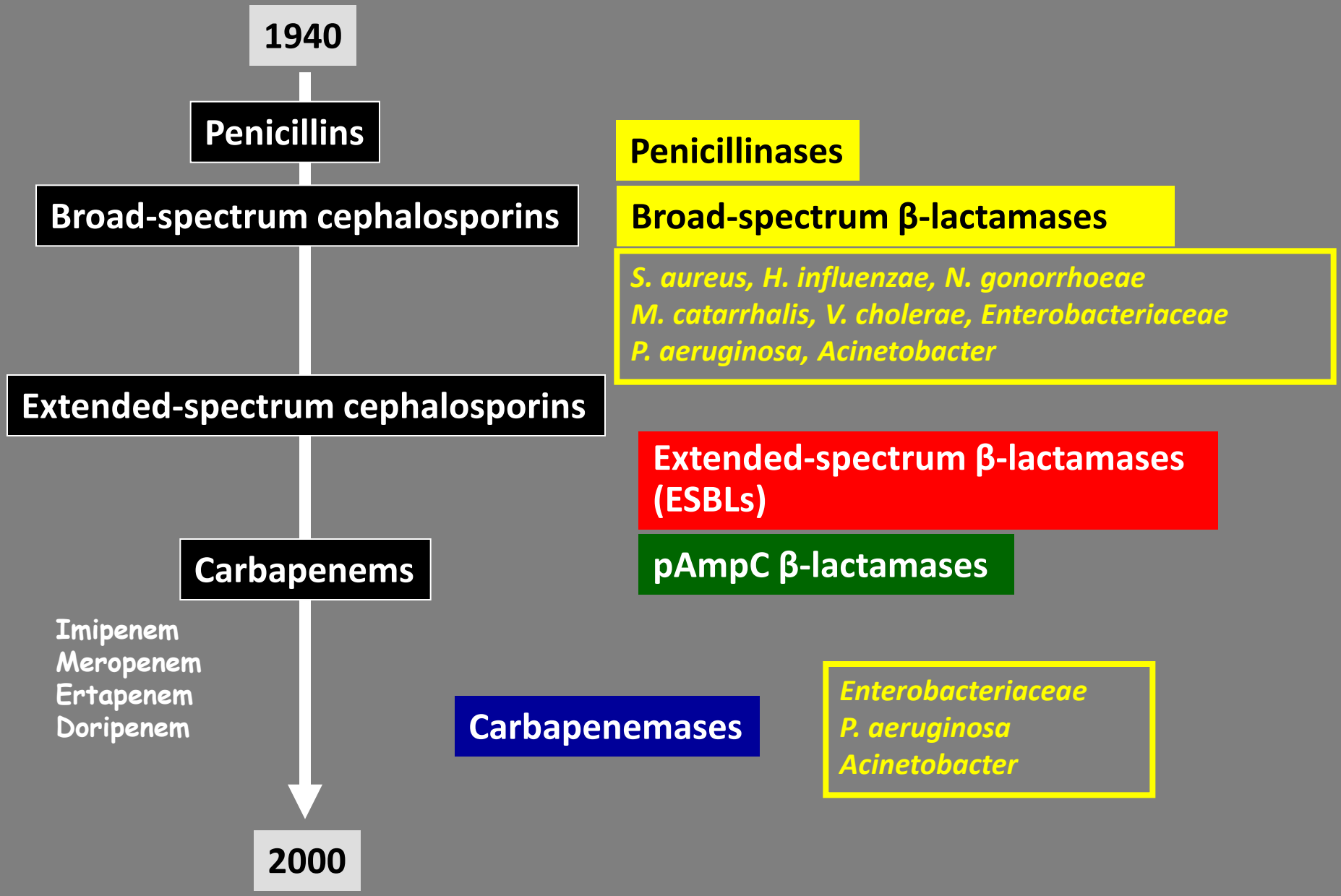
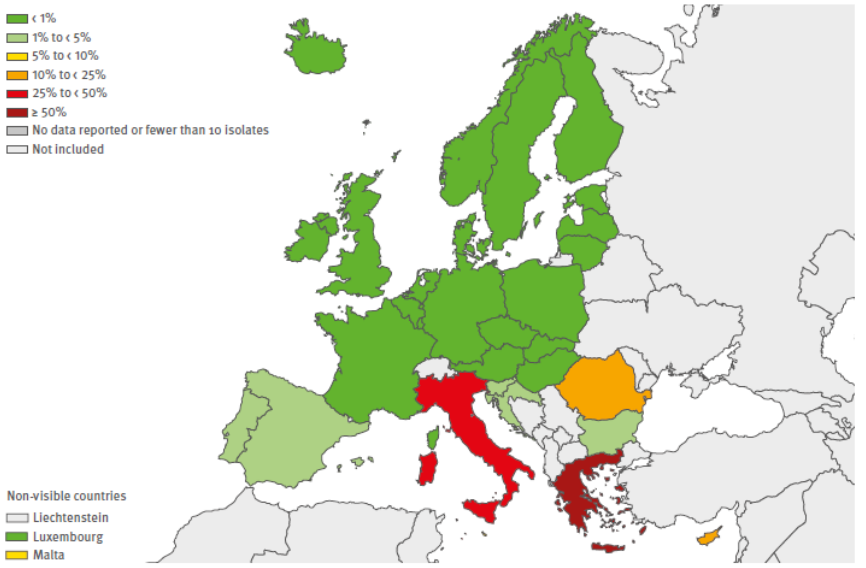

1940
Penicillins
Broad-spectrum cephalosporins
Extended-spectrum cephalosporins
Carbapenems
Imipenem
Meropenem
Ertapenem
Doripenem
2000
Penicillinases
Broad-spectrum β-lactamases
S. aureus, H. influenzae, N. gonorrhoeae
M. catarrhalis, V. cholerae, Enterobacteriaceae
P. aeruginosa, Acinetobacter
Extended-spectrum β-lactamases (ESBLs)
pAmpC β-lactamases
Carbapenemases
Enterobacteriaceae
P. aeruginosa
Acinetobacter

E. coli, 2015: R to carbapenems

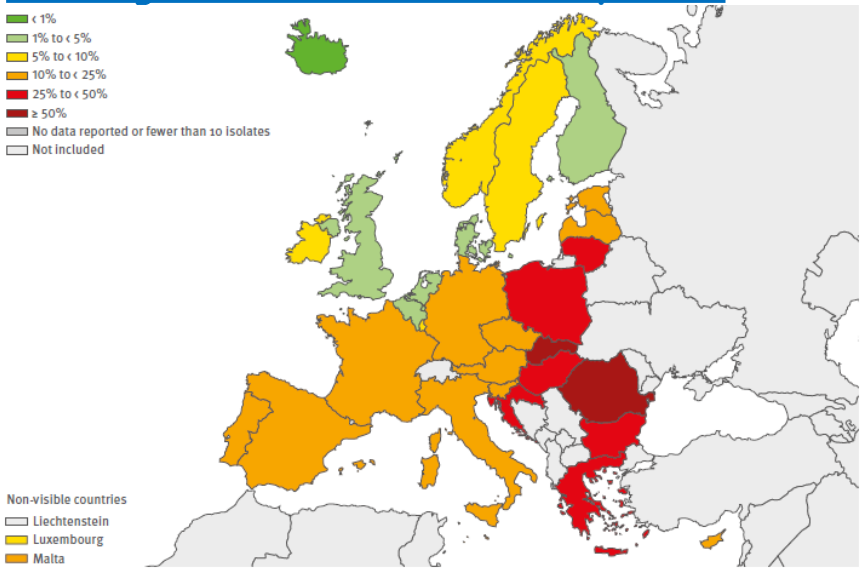
EU: 2012: 0.1% - 2015: 0.1%

K. pneumoniae, 2015: R to carbapenems



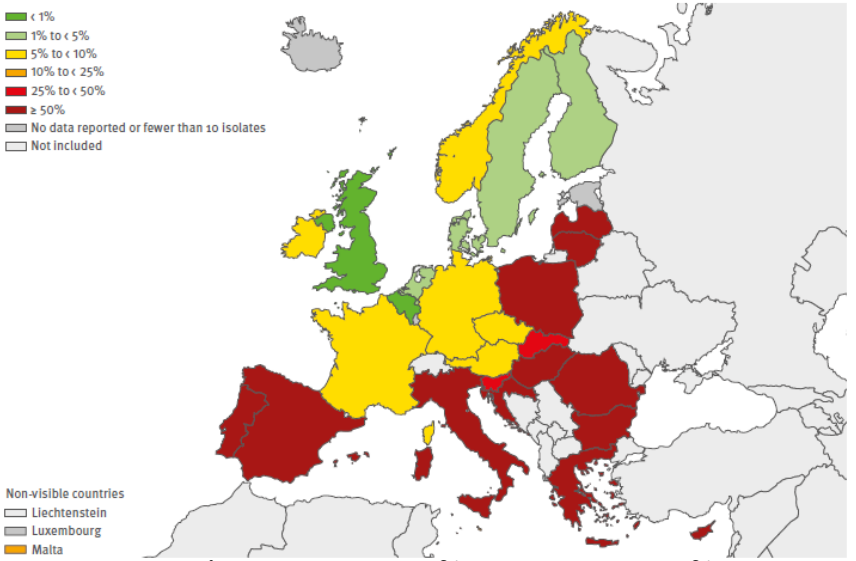
EU: 2012: 6.2% - 2015: 8.1%

P. aeruginosa, 2015: R to carbapenems



EU: 2012: 17.2% - 2015: 17.8%

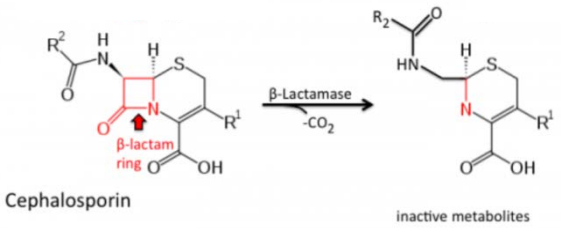
Acinetobacter spp., 2015: R to carbapenems



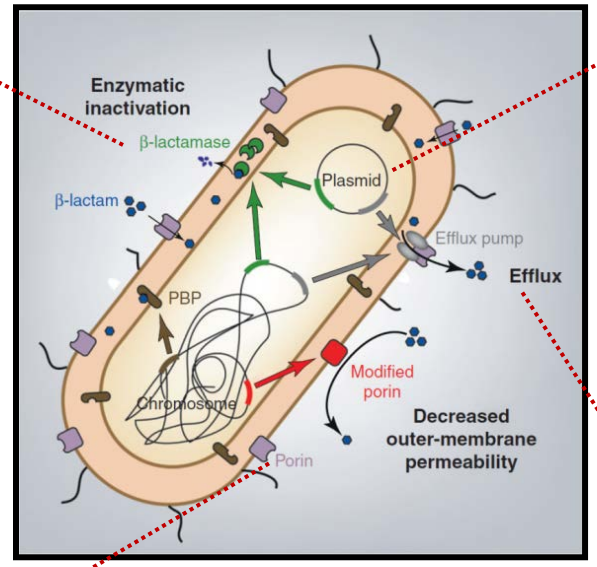
Italy: 2012: 83.1% - 2015: 78.3%

How they become resistant to β-lactams?

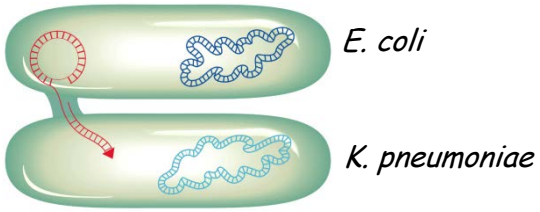
Inactivation of β-lactams



>2000 described (with very ≠ spectrum)



Plasmid conjugation



Exchange and spread among different bacterial species

Naturally present
(e.g., *P. aeruginosa* are R ertapenem)

Acquired
(chromosomal disruptions)

Naturally present
(e.g., *P. aeruginosa*)

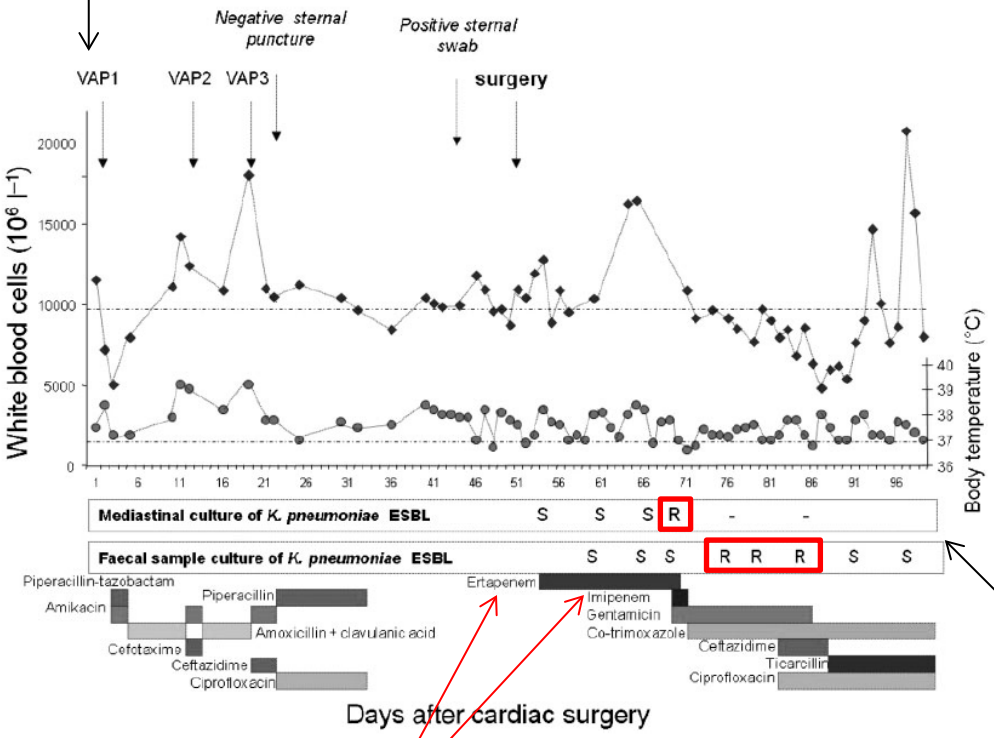
Acquired
(chromosomal overexpression)

Development of ertapenem resistance in a patient with mediastinitis caused by *Klebsiella pneumoniae* producing an extended-spectrum β-lactamase

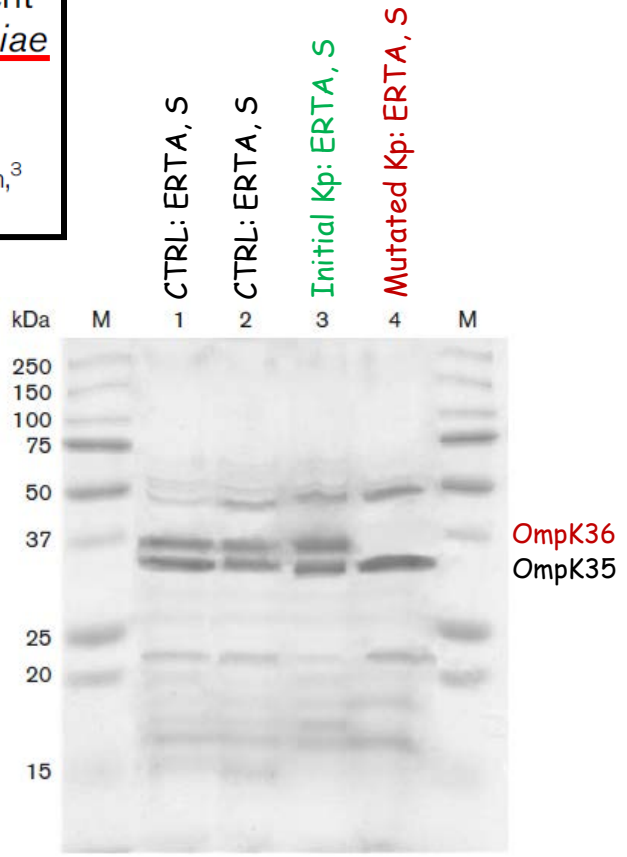
David Skurnik,[1]† Sigismond Lasocki,[2] Sylvie Bremont,[3] Claudette Muller-Serieys,[1] Marie Dominique Kitzis,[4] Patrice Courvalin,[3] Antoine Andremont[1] and Philippe Montravers[2]

JMM, 2010

Triple coronary bypass



ERTA: 1 g/day
IMP: 4 g/day



Identical by PFGE

IMP: from ≤0.125 to 8 mg/L
MEM: from ≤0.125 to 12 mg/L
ERTA: from 0.094 to 8 mg/L

MINIREVIEW
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2010, p. 969–976

Updated Functional Classification of β-Lactamases[▽]

Karen Bush[1]* and George A. Jacoby[2]

TABLE 1. Classification schemes for bacterial β-lactamases, expanded from Bush et al. (16)

Bush-Jacoby group (2009)	Bush-Jacoby-Medeiros group (1995)	Molecular class (subclass)	Distinctive substrate(s)	Inhibited by		Defining characteristic(s)	Representative enzyme(s)
				CA or TZB[a]	EDTA		
1	1	C	Cephalosporins	No	No	Greater hydrolysis of cephalosporins than benzylpenicillin; hydrolyzes cephamycins	*E. coli* AmpC, P99, ACT-1, CMY-2, FOX-1, MIR-1
1e	NI[b]	C	Cephalosporins	No	No	Increased hydrolysis of ceftazidime and often other oxyimino-β-lactams	GC1, CMY-37
2a	2a	A	Penicillins	Yes	No	Greater hydrolysis of benzylpenicillin than cephalosporins	PC1
2b	2b	A	Penicillins, early cephalosporins	Yes	No	Similar hydrolysis of benzylpenicillin and cephalosporins	TEM-1, TEM-2, SHV-1
2be	2be	A	Extended-spectrum cephalosporins, monobactams	Yes	No	Increased hydrolysis of oxyimino-β-lactams (cefotaxime, ceftazidime, ceftriaxone, cefepime, aztreonam)	TEM-3, SHV-2, CTX-M-15, PER-1, VEB-1
2br	2br	A	Penicillins	No	No	Resistance to clavulanic acid, sulbactam, and tazobactam	TEM-30, SHV-10
2ber	NI	A	Extended-spectrum cephalosporins, monobactams	No	No	Increased hydrolysis of oxyimino-β-lactams combined with resistance to clavulanic acid, sulbactam, and tazobactam	TEM-50
2c	2c	A	Carbenicillin	Yes	No	Increased hydrolysis of carbenicillin	PSE-1, CARB-3
2ce	NI	A	Carbenicillin, cefepime	Yes	No	Increased hydrolysis of carbenicillin, cefepime, and cefpirome	RTG-4
2d	2d	D	Cloxacillin	Variable	No	Increased hydrolysis of cloxacillin or oxacillin	OXA-1, OXA-10
2de	NI	D	Extended-spectrum cephalosporins	Variable	No	Hydrolyzes cloxacillin or oxacillin and oxyimino-β-lactams	OXA-11, OXA-15
2df	NI	D	Carbapenems	Variable	No	Hydrolyzes cloxacillin or oxacillin and carbapenems	OXA-23, OXA-48
2e	2e	A	Extended-spectrum cephalosporins	Yes	No	Hydrolyzes cephalosporins. Inhibited by clavulanic acid but not aztreonam	CepA
2f	2f	A	Carbapenems	Variable	No	Increased hydrolysis of carbapenems, oxyimino-β-lactams, cephamycins	KPC-2, IMI-1, SME-1
3a	3	B (B1)	Carbapenems	No	Yes	Broad-spectrum hydrolysis including carbapenems but not monobactams	IMP-1, VIM-1, CcrA, IND-1
		B (B3)					L1, CAU-1, GOB-1, FEZ-1
3b	3	B (B2)	Carbapenems	No	Yes	Preferential hydrolysis of carbapenems	CphA, Sfh-1
NI	4	Unknown					

OXA-24/40

NDMs

[a] CA, clavulanic acid; TZB, tazobactam.
[b] NI, not included.

Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study Grundmann et al., LID, Feb 2017

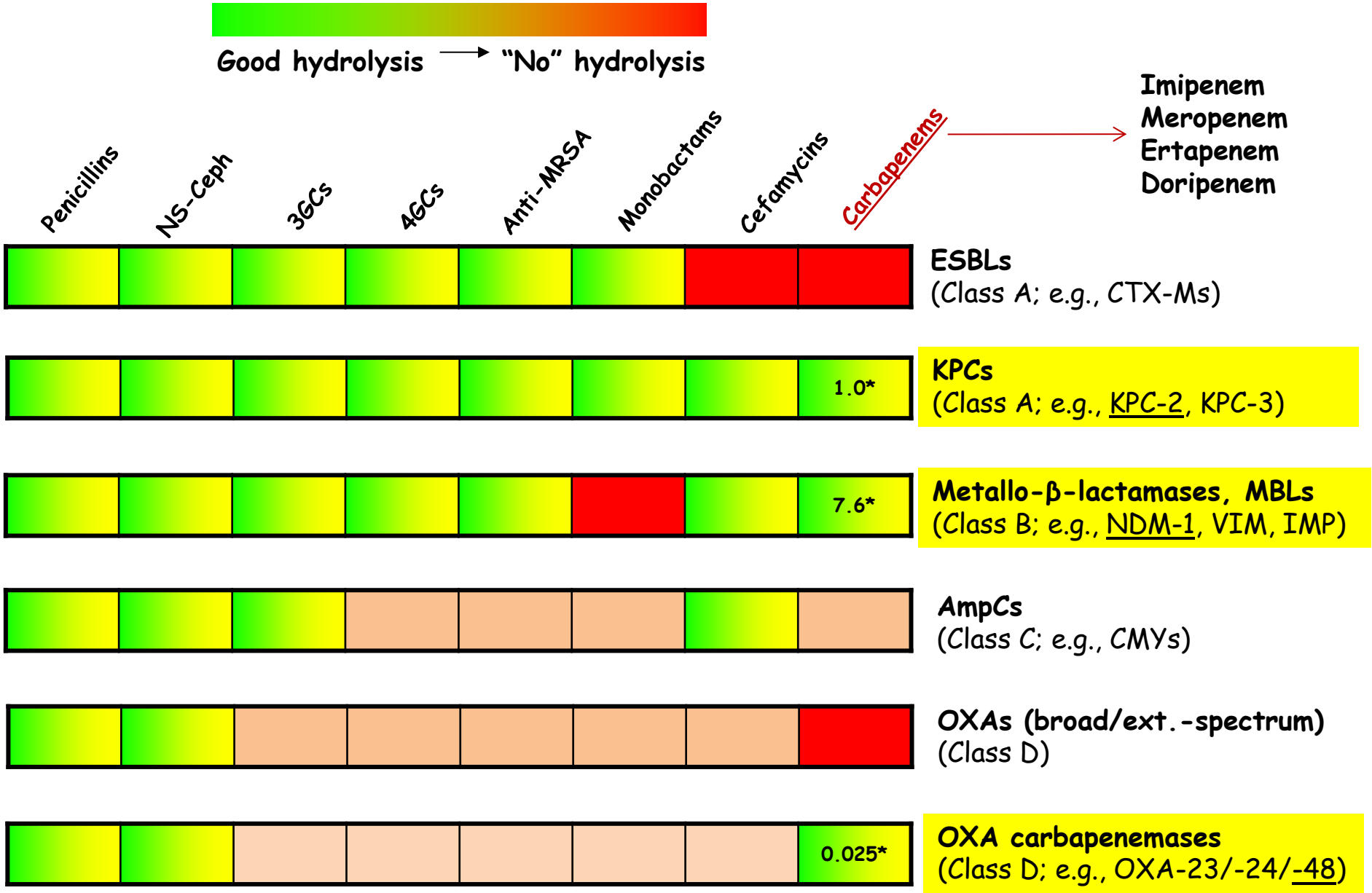
Nov, 2013 – Apr 2014
455 hospitals
36 countries

Klebsiella pneumoniae			*Escherichia coli*			Sentinel hospitals (mean beds†)	Incidence per 10000 admissions‡		Incidence per 100000 patient-days§	
Submitted non-susceptible isolates (n)	Confirmed carbapenemase-producing isolates* (n)	Comparator isolates (n)	Submitted non-susceptible isolates (n)	Confirmed carbapenemase producing isolates* (n)	Comparator isolates (n)		Rate (hospitals)	Rank	Rate (hospitals)	Rank
1203	850	1098	194	77	208	455 (800)	1·3 (321)	..	2·51 (268)	..
	70.6%			39.7%						

Hospitals submitting carbapenem non-susceptible *E coli* isolates (n)	Number of submitted carbapenem non-susceptible *E coli* isolates (n)	Confirmed carbapenemase-producing *E coli* isolates					Other (n, %)*
		KPC (n, %)	NDM (n, %)	OXA-48-like	VIM (n, %)	Total (n, %)	
105	194	14 (7·2)	20 (10·3)	43 (22·2)	0	77 (39·7)	117 (60·3)
		18.2%	26.0%	55.8%			

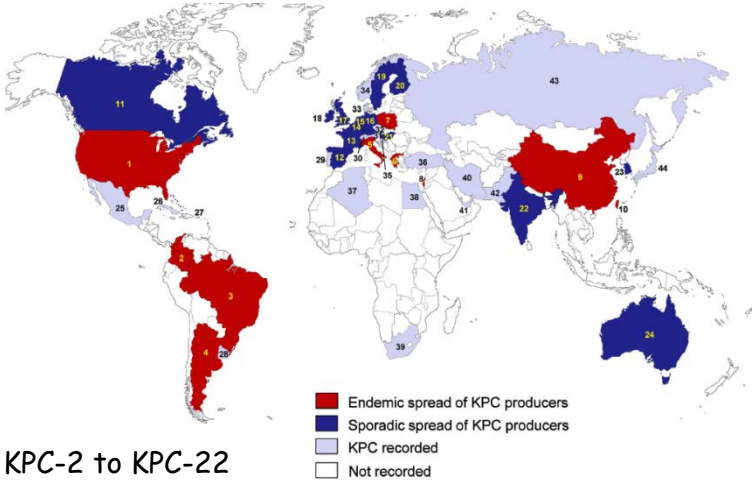
Hospitals submitting carbapenem non-susceptible *K pneumoniae* isolates (n)	Number of submitted carbapenem non-susceptible *K pneumoniae* isolates	Confirmed carbapenemase-producing *K pneumoniae* isolates					Other (n, %)*
		KPC (n, %)	NDM (n, %)	OXA-48-like (n, %)	VIM (n, %)	Total (n, %)	
251	1203	379 (31·5)	93 (7·7)	310 (25·8)	68 (5·7)	850 (70·7)	353 (29·3)
		44.6%	10.9%	36.5%	0.08%		

1. KPC: 42.4%
2. OXA-48-like: 38.1%
3. NDM: 12.2%
4. VIM: 7.3%



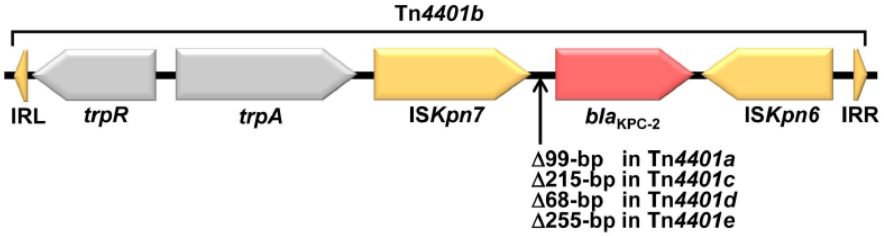
*Catalytic efficiency for imipenem: $kcat/K_m$ [$\mu M^{-1}/s^{-1}$]

KPC carbapenemases (class A)

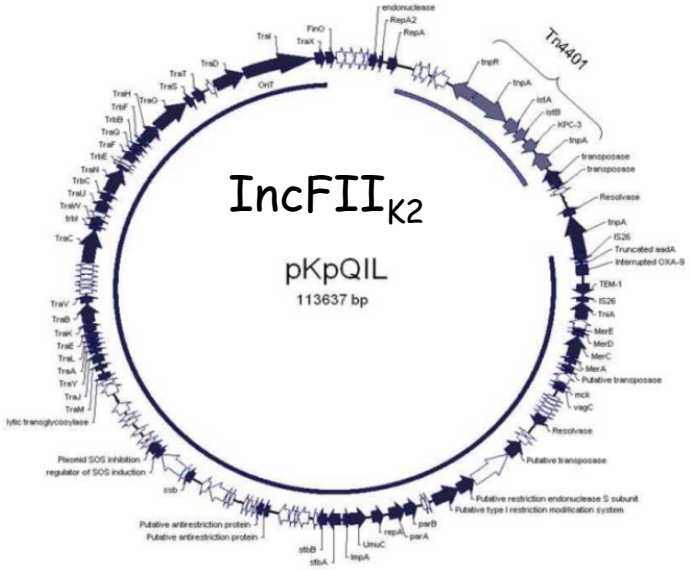


KPC-2 to KPC-22

The Tn4401 transposon (10 Kb) is at the origin of acquisition and dissemination of *bla*$_{KPC}$



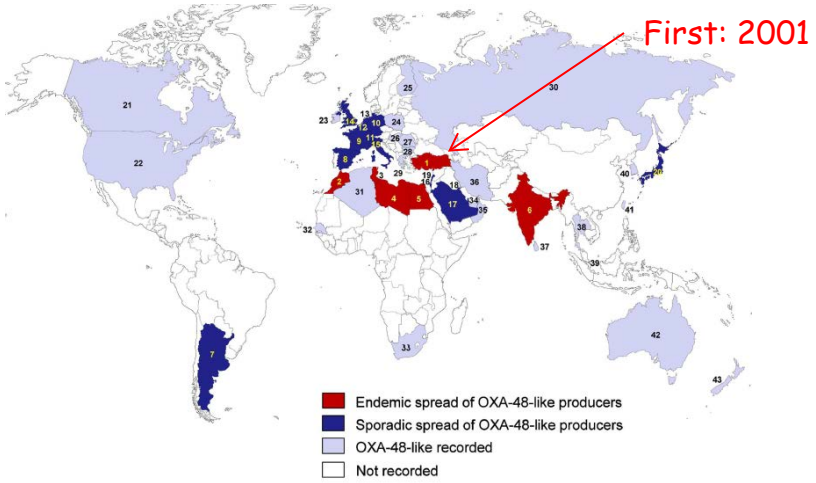
Tn4401 is carried by MDR plasmids (IncF, I2, X, A/C, R, ColE1) of which some hyperepidemic



Tn4401/plasmids are carried by *K. pneumoniae* hyperepidemic clones

MLST	*gapA*	*infB*	*mdh*	*pgi*	*phoE*	*rpoB*	*tonB*
ST11	3	3	1	1	1	1	4
ST37	2	9	2	1	13	1	16
ST258	3	3	1	1	1	1	79
ST277	3	1	1	1	1	1	43
ST307	4	1	2	52	1	1	7
ST327	2	1	1	1	10	1	19
ST340	3	3	1	1	1	1	18
ST376	2	6	1	3	8	1	27
ST384	18	23	56	63	80	43	51
ST388	16	24	59	27	29	22	105
ST512	54	3	1	1	1	1	79

OXA-48-like carbapenemases (class D)

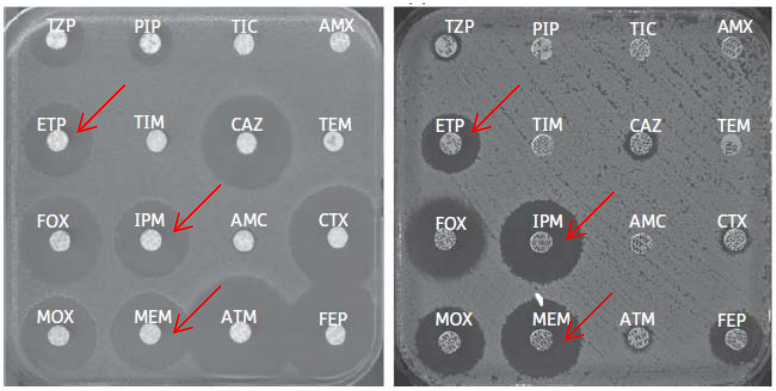


OXA-48 variants: OXA-162, -163, -181, -204, -232, -244, -245

OXA-48-like carbapenemases: the phantom menace

J Antimicrob Chemother 2012; **67**: 1597–1606

Laurent Poirel*, Anaïs Potron and Patrice Nordmann

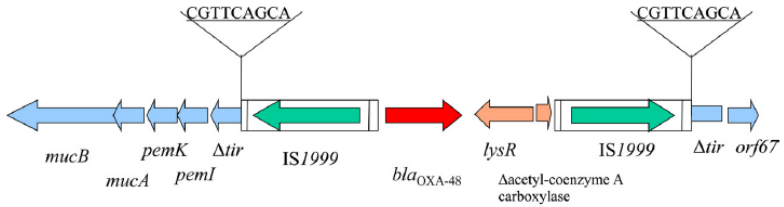


OXA-48 *K. pneumoniae* ESBL/OXA-48 *K. pneumoniae*

Common hyperepidemic L plasmid (non-MDR)

Strain	Country	Size (kb)[a]	Plasmid feature			Inc group	Plasmid-associated resistance marker
			RepA	TraU	ParA		
K. pneumoniae 11978	Turkey	ca. 62	+	+	+	L/M	None
K. pneumoniae Bel	Belgium	ca. 62	+	+	+	L/M	None
K. pneumoniae Lib	Lebanon	ca. 62	+	+	+	L/M	None
K. pneumoniae 8	Turkey	ca. 62	+	+	+	L/M	None
K. pneumoniae Egy	Egypt	ca. 62	+	+	+	L/M	None
K. pneumoniae 5A	Turkey	ca. 62	+	+	+	L/M	None
K. pneumoniae 7A	Turkey	ca. 62	+	+	+	L/M	None
K. pneumoniae Bey	Lebanon	ca. 62	+	+	+	L/M	None
K. pneumoniae 3A	Turkey	ca. 62	+	+	+	L/M	None
K. pneumoniae 4A	Turkey	ca. 62	+	+	+	L/M	None
K. pneumoniae 17A	Turkey	ca. 62	+	+	+	L/M	None
K. pneumoniae Bou	France	ca. 62	+	+	+	L/M	None
K. pneumoniae Dia	France	ca. 62	+	+	+	L/M	None
K. pneumoniae C2	Morocco	ca. 62	+	+	+	L/M	None
K. oxytoca A7	Morocco	ca. 62	+	+	+	L/M	None
E. coli 1	Turkey	ca. 62	+	+	+	L/M	None
E. cloacae 501	France	ca. 62	+	+	+	L/M	None
E. cloacae Bou	Morocco	ca. 62	+	+	+	L/M	None
E. cloacae D4	Morocco	ca. 62	+	+	+	L/M	None

Composite transposon Tn*1999*

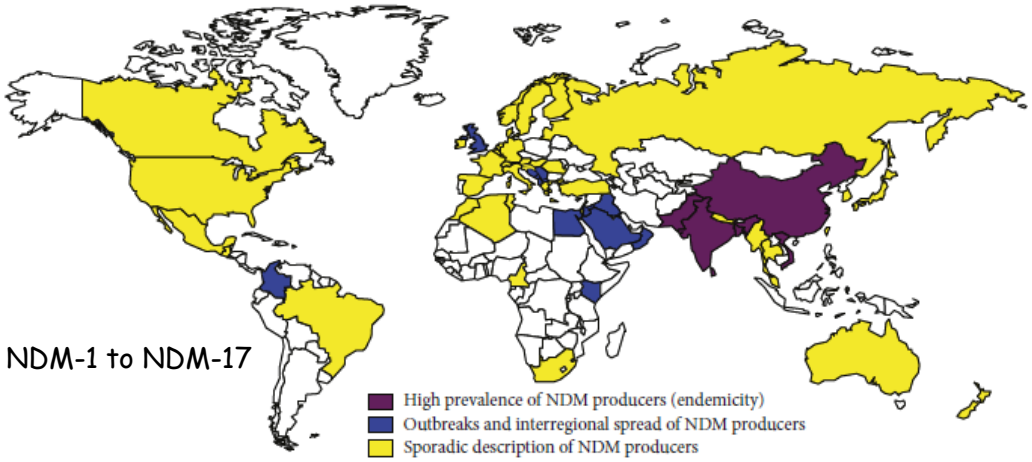


Hyperepidemic clones

E. coli ST131

K. pneumoniae ST11, ST147

NDM carbapenemases (class B, MBL)



Characterization of a New Metallo-β-Lactamase Gene, *bla*$_{NDM-1}$, and a Novel Erythromycin Esterase Gene Carried on a Unique Genetic Structure in *Klebsiella pneumoniae* Sequence Type 14 from India[▽]

Dongeun Yong,[1,2] Mark A. Toleman,[2] Christian G. Giske,[3] Hyun S. Cho,[4] Kristina Sundman,[5] Kyungwon Lee,[1] and Timothy R. Walsh[2]*

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2009, p. 5046–5054

2008: male with UTI/colonized (back from India)

Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study *Lancet Infect Dis 2010;*

2009: many UK patients who traveled in India/Pakistan

Very promiscuous *bla* gene(s)

Many species

2009-2012 (950 NDM strains)

K. pneumoniae	37.8%
E. coli	28.2%
Other Gram-neg. bacilli	12.0%
Other *Enterobacteriaceae*	11.8%
E. cloacae	5.5%
A. baumannii	3.8%
P. aeruginosa	1.0%

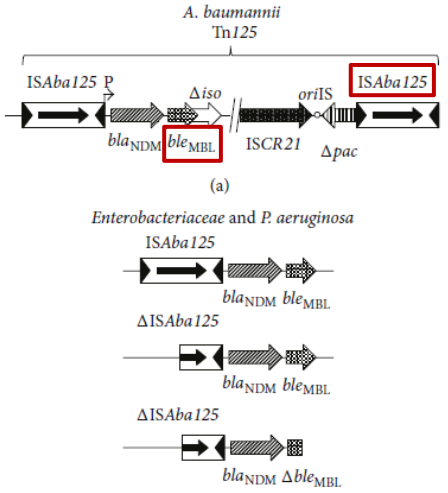
Not necessarily hyperepidemic clones!

Many MDR plasmids

IncA/C, IncF, IncR, IncH, IncN, IncX, IncL/M

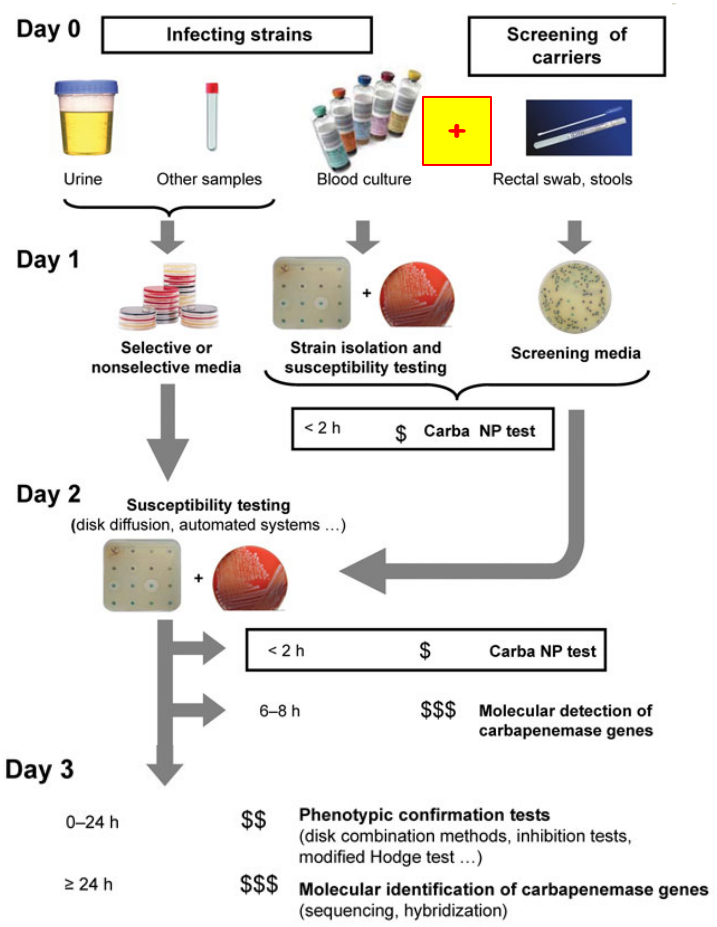
Frequently co-carrying important ARGs (e.g., 23S rRNA methylases)

Genetic structures



Berrazeg M., Eurosurveillance, 2013 – Dortet L., BioMedRes Int, 2014

Standard culture approach



Even 5-6 days to get the final report

Empirical treatment

Isolation

Directed treatment

Outcome

Septic shock

Inappropriate antibiotics within the first 6 hours is associated with a 5-fold higher mortality risk

[Kumar A *et al.*, Chest, 2009]

The Changing Role of the Clinical Microbiology Laboratory in Defining Resistance in Gram-negatives IDCNA, 2016

Andrea Endimiani, MD, PhD[a,*], Michael R. Jacobs, MD, PhD[b]

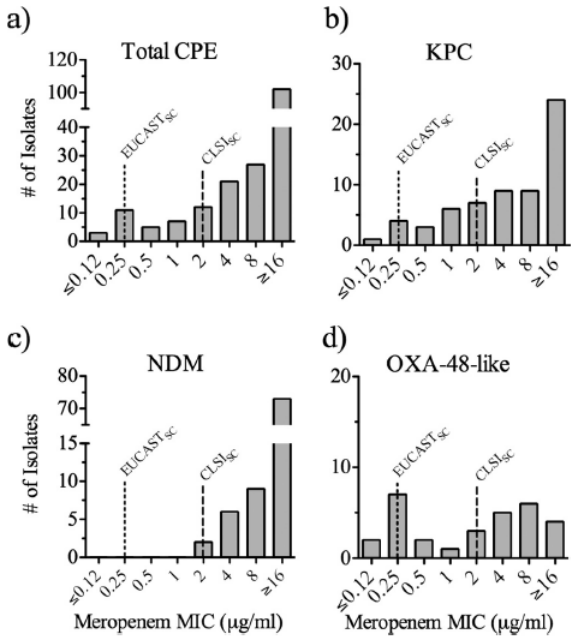
CLSI 2017: M100-S27

Laboratories using *Enterobacteriaceae* MIC **breakpoints** for carbapenems described in M100-S20 (January 2010) should perform the MHT, the Carba NP test, **mCIM,** and/or a molecular assay when isolates of *Enterobacteriaceae* are suspicious for carbapenemase production based on imipenem or meropenem MICs of 2–4 µg/mL or ertapenem MIC of 2 µg/mL (refer to Tables 3B, 3C, **and 3D**). After implementation of the current **breakpoints, these additional tests do** not need to be performed other than for epidemiological or infection control purposes (refer to Table 3B).

	CLSI, Jan 2010		CLSI, 2017		EUCAST, 2017	
	S	R	S	R	S [screening]	R
IMP	≤4	≥16	≤1	≥4	≤2 [>1]	≥16
MEM	≤4	≥16	≤1	≥4	≤2 [>0.12]	≥16

Screening tests

MHT (Table 3B)	Carba NP (Table 3C)	mCIM (Table 3D)
Enterobacteriaceae that are not susceptible to one or more carbapenems	*Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter* spp. that are not susceptible to one or more carbapenems	***Enterobacteriaceae* that are not susceptible to one or more carbapenems**



What Is the Appropriate Meropenem MIC for Screening of Carbapenemase-Producing *Enterobacteriaceae* in Low-Prevalence Settings? Fattouh R et al., AAC, March 2016

1,022 *Enterobacteriaceae* (189 CPE)

CLSI:
- 14% had MICs in the S range
- Screening tests (if used) captured 86% of the CPE

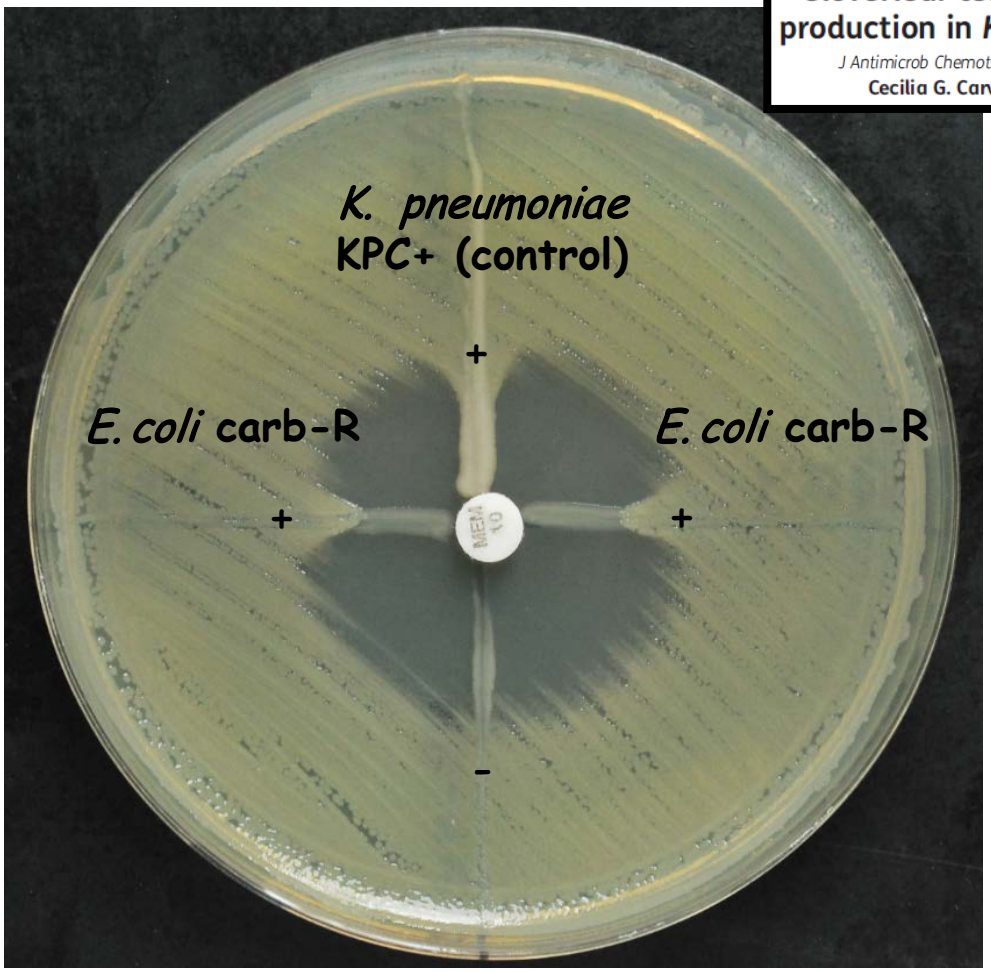
EUCAST:
- 20% had MICs in the S range
- Screening tests captured 98% of the CPE

CLSI: Modified Hodge test

Cloverleaf test (modified Hodge test) for detecting carbapenemase production in *Klebsiella pneumoniae*: be aware of false positive results

J Antimicrob Chemother 2010; **65**: 249–251

Cecilia G. Carvalhaes*, Renata C. Picão, Adriana G. Nicoletti, Danilo E. Xavier and Ana C. Gales



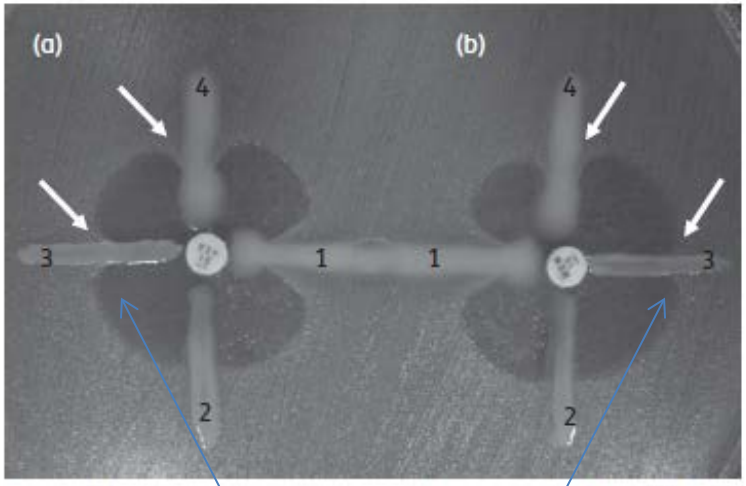


Figure 1. Phenotypic carbapenemase detection by MHT applying the inoculum recommended by CLSI for ertapenem (a) and meropenem discs (b). 1, *K. pneumoniae* ATCC BAA-1705, positive result; 2, *K. pneumoniae* ATCC BAA-1706, negative result; 3, CTX-M-producing *K. pneumoniae* clinical isolate; 4, KPC-producing *K. pneumoniae* clinical isolate. Arrows indicate the similar size of *E. coli* ATCC 25922 grown within the carbapenem disc inhibition zones when testing both carbapenemase producer and carbapenemase non-producer isolates.

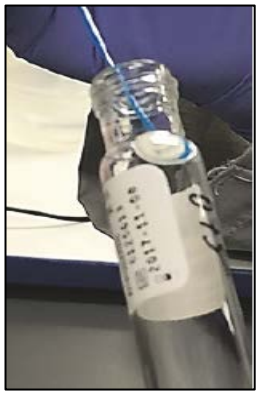
This test indicates the possible production of enzyme(s) able to hydrolyze carbapenems (i.e., meropenem) = generic CARBAPENEMASE

ESBL or pAmpC producers with porin(s) loss

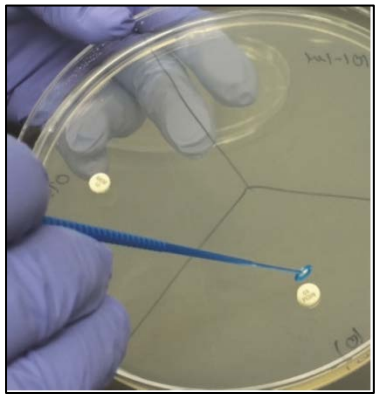
Not good for NDMs

CLSI: Modified Carbapenem Inactivation Method (mCIM)

1 µL loop bacteria
2 mL TSB
10µg MEM

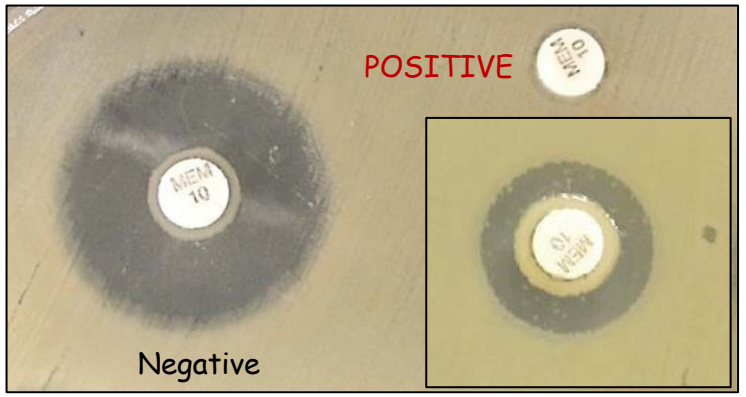


4 h incubation



ATCC 25922 *E. coli*
0.5 McFarland
MH agar

35°C±2
18-24 h



6-15 mm: Carbapenemase positive
16-18 mm: Indeterminate results
≥ 19 mm: No carbapenemases

Modified Carbapenem Inactivation Method for Phenotypic Detection of Carbapenemase Production among *Enterobacteriaceae* Pierce VM, JCM, Aug 2017

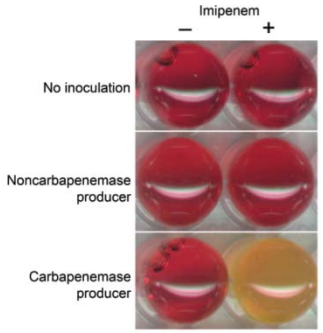
117 *Enterobacteriaceae* (94 CPE)

Single clinical laboratory
Sens: 99%; Spec: 100%

Nine laboratory study
Sens: 97%; Spec: 99%

Rapid Detection of Carbapenemase-producing *Enterobacteriaceae*

Nordmann, EID, 2012



CLSI: NP test

<2 hrs
Most CPE turned in 10-30 min

!!!! OXA-48-like
not always detected

Need pre-extraction of enzymes (~30 min)

Blue-Carba, an Easy Biochemical Test for Detection of Diverse Carbapenemase Producers Directly from Bacterial Cultures
J. Pires, A. Novais, L. Peixe December 2013 Volume 51 Number 12 Journal of Clinical Microbiology

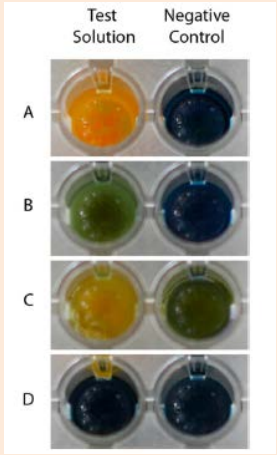
Bromothymol blue

A: NDM-1 *E. coli*

B: OXA-23 *A. baumannii*

C: OXA-48 *K. pneumoniae*

D: *E. coli* ATCC 25922



Direct use of colonies!

Non-molecular detection of carbapenemases in Enterobacteriaceae clinical isolates

A. Aguirre-Quiñonero [a], L. Martínez-Martínez [a, b, *] J Infect Chemother 23 (2017) 1–11

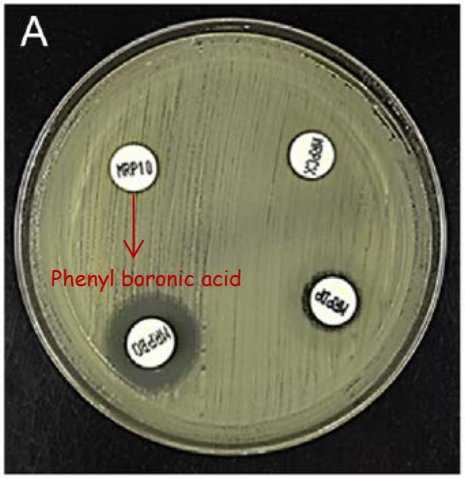
Inhibitor based approaches

	Synergy with MPM			
	DPA EDTA	PBA	DPA + PBA	Cloxacillin
KPC	–	+	–	–
MBL	+	–	–	–
OXA-48-like	–	–	–	–
KPC + MBL	v	v	+	–
AmpC + porin loss	–	+	–	+
ESBL + porin loss	–	–	–	–

MPM: Meropenem, DPA: dipicolinic acid, PBA: phenyl boronic acid.

Combination disk test (CDT)

Rosco Diagnostica KPC/MBL confirmation kit

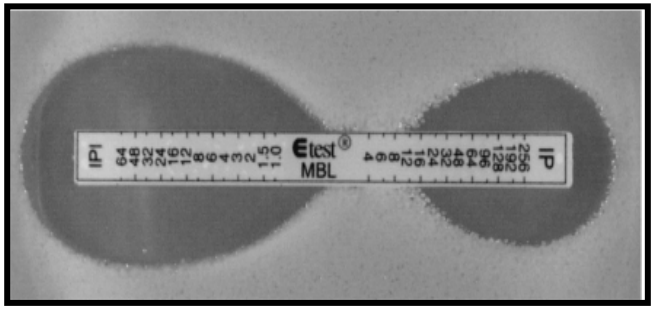


KPC-producing *K. pneumoniae*

NDM-producing *K. pneumoniae*

Gradient diffusion strips

Etest: Imipenem+EDTA / Imipenem



VIM-producing *K. pneumoniae*

Evaluation of a DNA microarray for rapid detection of the most prevalent extended-spectrum β-lactamases, plasmid-mediated cephalosporinases and carbapenemases in Enterobacteriaceae, *Pseudomonas* and *Acinetobacter* Bogaerts P, IJAA, 2016

50-85 Euro

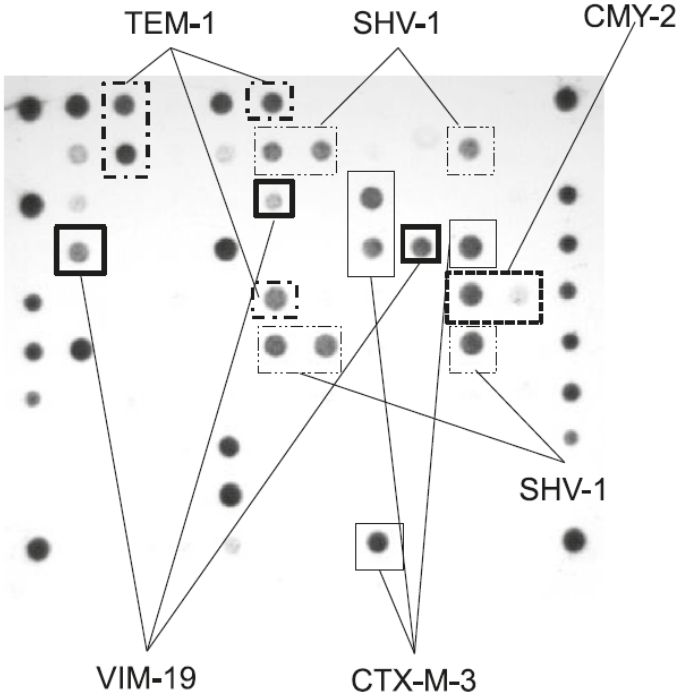
~6 hrs

ESBLs: TEM, SHV, CTX-M, BEL, PER, GES, VEB

pAmpCs: CMY, DHA, FOX, ACC-1, ACT/MIR

Carbapenemases: KPC, NDM, VIM, IMP, GIM, SPM, OXA-48, OXA-23, OXA-24, OXA-58

HybC	TEM 104E (1)	TEM 104K (2)	DNA-C (3)	TEM 164R (4)	TEM 164S (5)		TEM 164H (7)	IMP (8)	IMP (9)
CTR (10)	TEM 238G (11)	TEM 238S (12)	DNA-C (13)	SHV-all (14)	SHV 238G (15)	SHV G238S (16)	SHV G238A (17)	SHV 240E (18)	SHV E240K (19)
CTR (20)	OXA-48 (21)	OXA-48 (22)	negC (23)	VIM (24)	VIM (25)	CTX-M1 fam (26)	CTX-M2 fam (27)	CTX-M9 fam (28)	CTX-M8+25 fam (29)
VIM-all (30)	KPC (31)	KPC (32)	HybC	FOX (33)	CTX-M1-A (34)	CTX-M1-B (35)	VIM-all (36)	CTX-M1-C (37)	CTX-M1-D (38)
PER I (39)	NDM (40)	PER I (41)	PER II (42)	TEM -all (43)	TEM 164S (44)	TEM 164C (45)	TEM 164H (46)	CMY II (47)	CMY II (48)
HybC	NDM (49)	VEB (50)	VEB (51)	SHV-all (52)	SHV 238G (53)	SHV G238S (54)	SHV G238A (55)	SHV 240E (56)	SHV E240K (57)
GIM (58)	DHA (59)	ACT/MIR (60)	GIM (61)	CMY I / MOX (62)	ACC (63)	SPM (64)	SPM (65)	BEL (66)	BEL (67)
OXA-23 (68)	OXA-23 (69)	TEM 104K (70)	DNA-C (71)	OXA-24 (72)	OXA-24 (73)	OXA-58 (74)	OXA-58 (75)	IMP (76)	IMP (77)
negC (78)	GES-all (79)	TEM 238S (80)	HybC	GES CARBA (81)	GES ESBL (82)	*mcr*	*mcr*	*mcr*	
	DHA (88)	ACT/MIR (89)	DNA-C (90)	CMY I / MOX (91)	ACC (92)	CTX-M1 fam (93)	CTX-M2 fam (94)	CTX-M9 fam (95)	CTX-M8+25 fam (96)



Sensitivity and Specificity: both ~100%

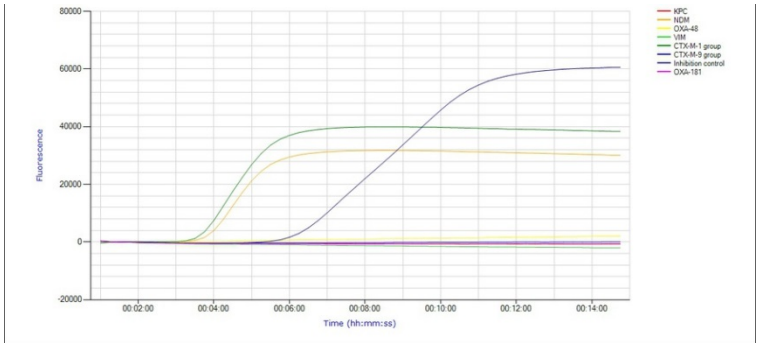
Amplex Eazyplex

Genie II platform

- Preparation (**5 min**)
- No DNA extraction
- Run time (**<30 min**)

~55 Euro

LAMP
Loop-mediated isothermal Amplification
Real-time fluorescent measurement



eazyplex® SuperBug CRE

KPC,
NDM,
OXA-48
and OXA 181,
VIM, as well as
CTX-M-1 and
CTX-M-9 group

eazyplex® SuperBug mcr-1

eazyplex® SuperBug complete

Version A	Version B
KPC	KPC
NDM	NDM
OXA-48	OXA-48
VIM	VIM
OXA-23 group	OXA-23 group
OXA-40 group	OXA-40 group
OXA-58 group	OXA-181

Evaluation of the eazyplex® SuperBug CRE system for rapid detection of carbapenemases and ESBLs in clinical Enterobacteriaceae isolates recovered at two Spanish hospitals

J Antimicrob Chemother 2015; **70**: 1047–1050

94 carbapenemase-producing *Enterobacteriaceae*

100% agreement with PCR/Sequencing of *bla* genes

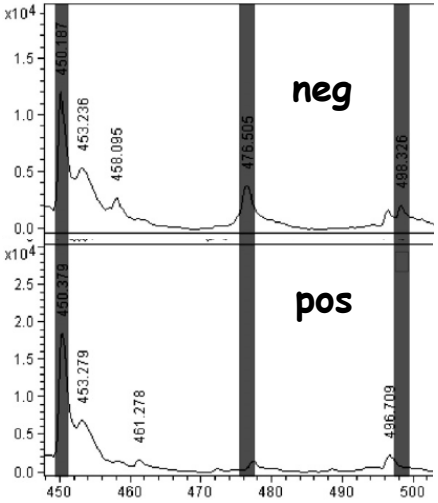
KPC-2	*K. pneumoniae* (2), *Enterobacter cloacae* (2), *E. coli* (1)
KPC-3	*K. pneumoniae* (22), *E. cloacae* (1), *E. coli* (2)
KPC-3+CTX-M-15	*K. pneumoniae* (1)
VIM-1	*K. pneumoniae* (2), *Klebsiella oxytoca* (1), *E. coli* (2), *E. cloacae* (3), *Citrobacter freundii* (1), *Serratia marcescens* (2), *Raoultella ornithinolytica* (1)
VIM-1+CTX-M-10	*K. oxytoca* (1)
VIM-1+CTX-M-15	*K. pneumoniae* (1), *K. oxytoca* (1)
VIM-1+CTX-M-32	*E. coli* (1)
VIM-1+CTX-M-14	*K. oxytoca* (1)
NDM-1	*Providencia rettgeri* (1)
NDM-5	*E. coli* (1)
OXA-48	*K. pneumoniae* (3), *K. oxytoca* (1), *E. coli* (2), *E. cloacae* (1), *Citrobacter koseri* (1), *Citrobacter braakii* (1)
OXA-48+CTX-M-15	*K. pneumoniae* (30), *E. coli* (2), *Enterobacter aerogenes* (1), *Citrobacter amalonaticus* (1)
OXA-48+CTX-M-14	*E. coli* (1)

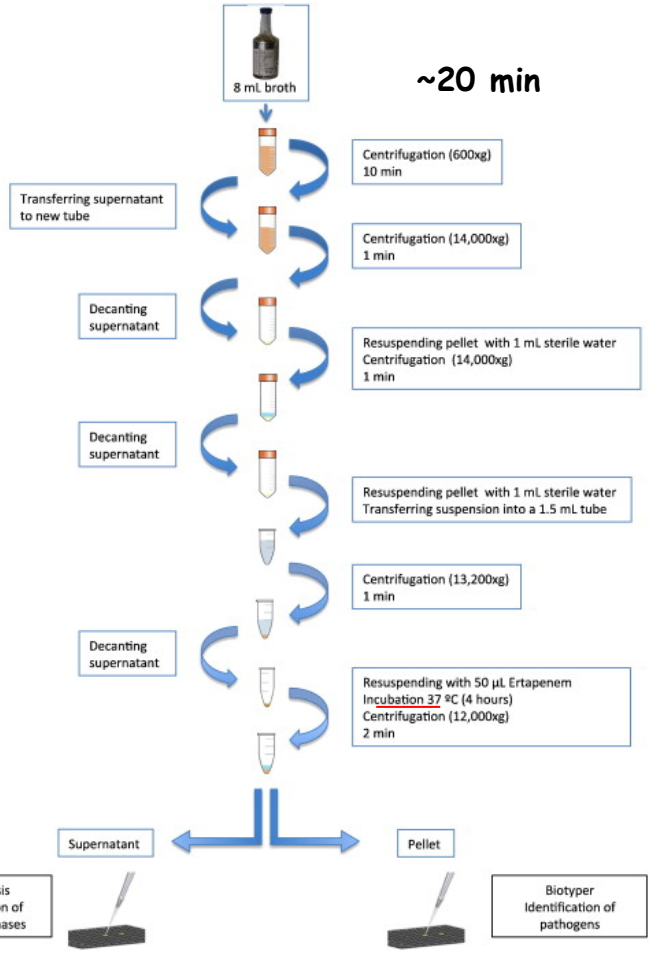
Rapid detection and identification of strains carrying carbapenemases directly from positive blood cultures using MALDI-TOF MS

Hoyos-Mallecot *et al.*, J Micro Meth; 105, 2014

19 carba producers (21 controls)
Sens and Spec: 100% and 90%

Ertapenem





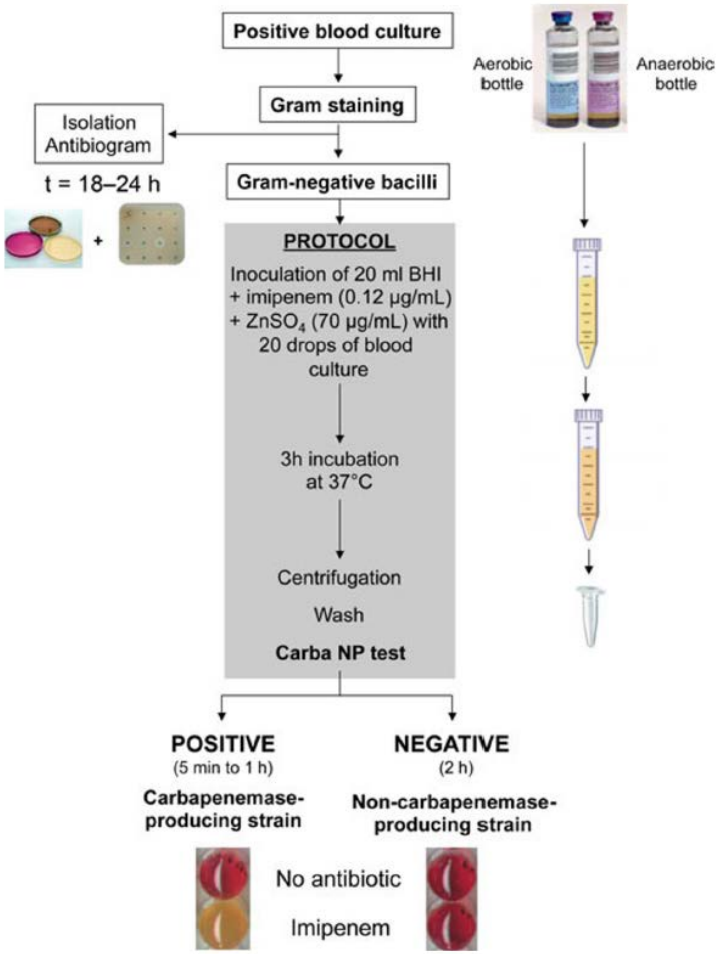
0.30 Euro*

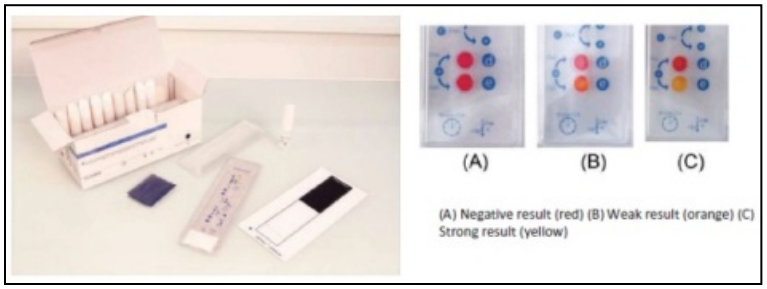
Generic activity in ~4.5 hrs
Can be reduced ~30 min-2 hrs

Rapid detection of carbapenemase-producing Enterobacteriaceae from blood cultures

L. Dortet[1], L. Bréchard[1], L. Poirel[1,2] and P. Nordmann[1,2]

Clin Microbiol Infect 2014; **20:** 340–344





The RAPIDEC Carba NP ®Test

Carbapenemase types	Tested isolates (*n*)	Carba NP test on positive blood culture: Positive *n*	Positive %	Negative *n*	Negative %	Sensitivity (%)	Specificity (%)
KPC	50	50	100	0	0	100	100
IMP	27	27	100	0	0	100	100
VIM	37	37	100	0	0	100	100
NDM	33	33	100	0	0	100	100
OXA-48-like	46	42	91.3	4	8.7	91.3	100
No carbapenemase	74	0	0	74	100	–	–
Total results						97.9	100

Preparation from +BCs: **~3 hrs**

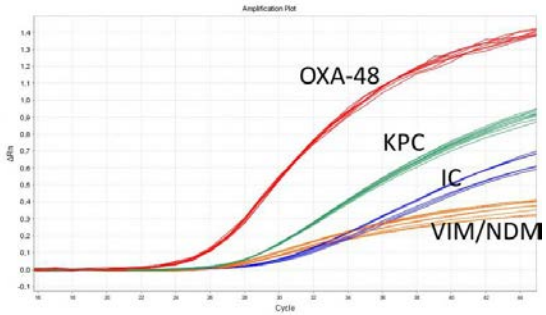
Reaction in 5 min-1 h (max 2 hrs)

Sens and Spec 98% and 100%

Check-Direct Screening, Check-Points

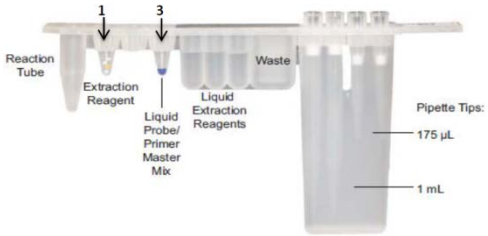
Rectal swab
(Copan, ESwab)

- Real-time multiplex PCR
- Rapid preparation
- Time to results (<3 hrs)



BD MAX

Reagent strip



Check-Direct CPE Screen for BD MAX™

~30 Euro

KPC
OXA-48-like
VIM
NDM

Performance of the BD MAX™ instrument with Check-Direct CPE real-time PCR for the detection of carbapenemase genes from rectal swabs, in a setting with endemic dissemination of carbapenemase-producing *Enterobacteriaceae* Antonelli *et al.*, DMID, 2016

Florence, Italy
557 rectal swabs

ChromID CARBA SMART (w/wo broth enrichment)
[5 samples not detected]

Limit of detection (LOD)

Target	LOD CPMix (CFU/ml)
bla_{KPC}	9×10^{3}
bla_{VIM}	4.5×10^{2}
bla_{OXA-48}	8.5×10^{1}
bla_{NDM}	7.3×10^{1}

Summary of the results

		Colonization status	
		Positive (n. 29)	*Negative (n. 528)*
CHSM and BERM	*Positive*	24 (82.8 %)[a]	0
	Negative	5 (17.2 %)	528 (100 %)
CPMix	*Positive*	29 (100 %)	15 (2.9 %)
	Negative	0	507 (96.0 %)
	Unresolved	-	6 (1.1 %)

23 KPC
5 VIM
1 OXA-48

TAT from 18-24 h (direct culture) or 48 h (broth enrichment) to 3 hrs

Cepheid GeneXpert

- Add aliquot to elution, vortex, transfer to port S
- Insert cartridge to station (overall, **1 min**)
- Run time (**<1 h**)

Real-time multiplex PCR

- Smart fluidic system
- Filtering and Sonication (DNA)
- Fluorescent-labeled hybr. probes (6 colors)
- Internal control

Xpert® Carba-R

KPC
NDM
OXA-48-like
VIM
IMP-1

~50 Euro

Multisite Evaluation of Cepheid Xpert Carba-R Assay for Detection of Carbapenemase-Producing Organisms in Rectal Swabs

JCM, 54:7; 2016

M. Tato,[a] P. Ruiz-Garbajosa,[a] M. Traczewski,[b] A. Dodgson,[c] A. McEwan,[c] R. Humphries,[d] J. Hindler,[d] J. Veltman,[e] H. Wang,[f] R. Cantón[a]

4 centers (2 USA, 1 UK, 1 Spain)
July 2013 – Feb 2014
633 samples

50% of them spiked at 1.1 x 10^2 to 1.2 x 10^3 CFU/swab (LOD of Xpert)

Results (obtained in 32-48 min)

Xpert Carba-R assay result	Clinical specimens (n = 383)	Contrived specimens (n = 250)	All specimens (n = 633)
Positive (single and/or combined targets)	42	107	149
IMP-1	0	25	25
VIM	2	24	26
NDM	2	23	25
KPC	13	19	32
OXA-48	20	15	35
VIM + OXA-48	4	0	4
NDM + KPC	1	0	1
IMP-1 + NDM	0	1	1
Negative	341	143	484

23.5%

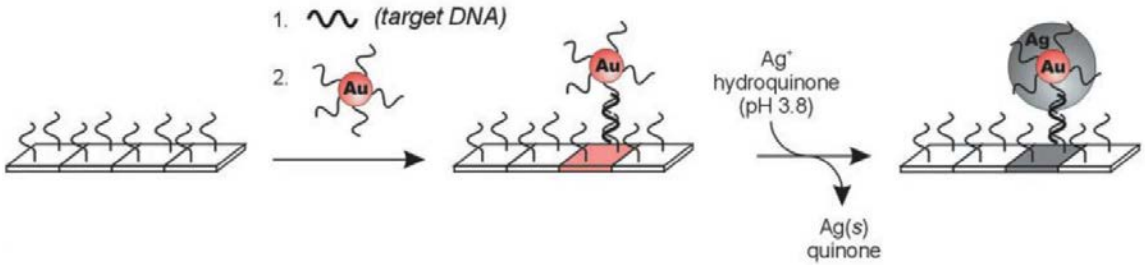
Performance for different targets

Target gene	Sensitivity (% [95% CI])	Specificity (% [95% CI])	PPV (%)	NPV (%)
IMP-1	96.3 (81.0–99.9)	100 (99.4–100)	100	99.8
VIM	93.5 (78.6–99.2)	99.8 (99.1–100)	96.7	99.7
NDM	100 (86.8–100)	99.8 (99.1–100)	96.3	100
KPC	96.7 (82.8–99.9)	99.3 (98.3–99.8)	87.9	99.8
OXA-48	95.0 (83.1–99.4)	99.8 (99.1–100)	97.4	99.7

Sensitivity: 96.6% - Specificity: 98.6%

Verigene, Nanosphere

- Load cartridge, consumables, and sample (**5 min**)
- Automated sample preparation and processing
- Place slide from cartridge in reader (**2.5 hrs**)



Microarray approach by using Au-nanoprobe as reporter and silver reduction to enhance signal

Gram-negatives cartridge

Species	Genus	Resistance
Escherichia coli	*Acinetobacter* spp.	CTX-M (ESBL)
Klebsiella pneumoniae	*Citrobacter* spp.	IMP (carbapenemase)
Klebsiella oxytoca	*Enterobacter* spp.	KPC (carbapenemase)
Pseudomonas aeruginosa	*Proteus* spp.	NDM (carbapenemase)
Serratia marcescens		OXA (carbapenemase)
		VIM (carbapenemase)

~50 Euro

Performance Evaluation of the Verigene Gram-Positive and Gram-Negative Blood Culture Test for Direct Identification of Bacteria and Their Resistance Determinants from Positive Blood Cultures in Hong Kong

Gilman K.H. Siu *et al.*, Plos One, Oct 2015

Multicenter study (4 hospitals)
364 BCs (114 Gram-pos; 250 Gram-neg)
Jan 2014 – May 2014

ID for Gram-negatives (agreement 90.5%)

Organisms	No. (%) of isolates					Sensitivity (%)	Specificity (%)
	Total	Correctly identified	Not detected	Misidentified	No Call		
E.coli	165 (59.1)	158 (95.8)	6 (3.6)	1[c]	-	95.8	100
K. pneumoniae	52 (18.6)	36 (69.2)	14 (27)[d]	2[e]	-	69.2[d]	100
P. aeruginosa	17 (6.1)	13 (76.5)	3 (17.6)	-	1	81.3	100
Proteus spp.	10 (3.6)	9 (90)	-	-	1	100	100
Enterobacter spp.	10 (3.6)	8 (80)	-	-	2	100	99.3
Acinetobacter spp.	4 (1.4)	4 (100)	-	-	-	100	100
K. oxytoca	3 (1.1)	-	3 (100)	-	-	0	99.3

K. variicola 80%

40-99 h faster than routine

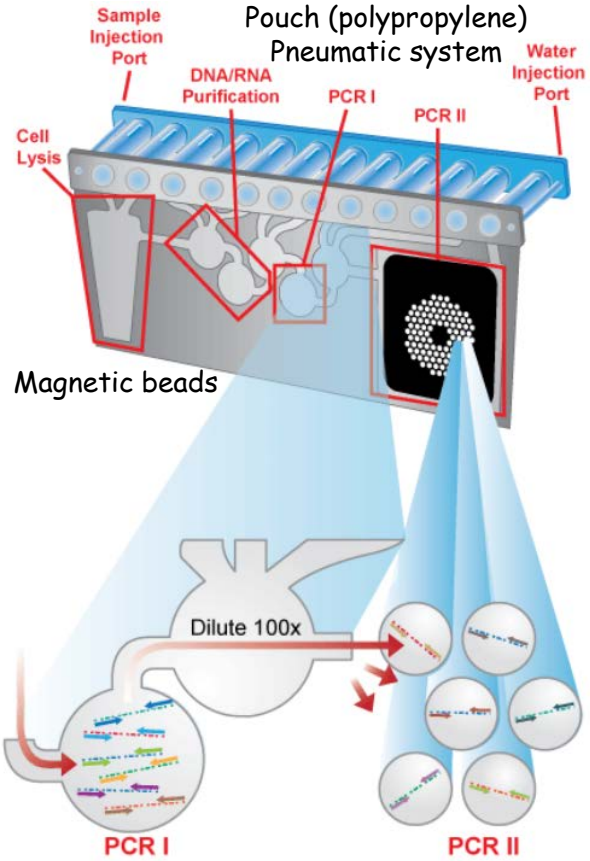
Organisms	No. of Isolates[b]	Average Time to identification by Culture-Based Method (h)	Average Time to Result by Verigene Test (h)	Δ Time to Result[a] Average (h)
Staphylococcus spp.	23	63.76	2.35	61.41
MSSA	7	49	2.35	46.65
MRSA	10	93.45	2.35	91.1
CNS	8	57.19	2.35	54.84
Streptococcus spp.	13	63.86	2.35	61.51
S. pneumoniae	3	42.83	2.35	40.48
β-haemolytic Strept.	4	71.62	2.35	69.27
Viridians Group	6	55.08	2.35	52.73
Enterococcus spp.	2	78.5	2.35	76.15
VSE	1	55.5	2.35	53.15
VRE	1	101.5	2.35	99.15
Enterobacteriaceae	79	45.98	1.88	44.1
P. aeruginosa	7	52.51	1.88	50.63
Acinetobacter spp.	1	76	1.88	74.12

Drug-Resistant Organisms

Drug resistant Organisms	No. of isolates				Sensitivity	Specificity (%)
	Total	Correctly Detected	Not Detected	No Call		
Gram Positive						
MRSA	27	26	0	1	100	100
MRSE	4	4	0	0	100	100
VRE	1	1	0	0	100	100
Gram Negative						
Cefotaxime resistant *Enterobacteriaceae* (including ESBL producers)	61	38	22	1	63.3	100
ESBL producing *Enterobacteriaceae*	46	38	7	1	84.4	100
MDR *Acinetobacter*	3	3	0	0	100	100
Carbapenem-resistant *Psuedomonas*	2	0	2	0	0	100
Total	**98**	**72**	**24**	**2**	**75.0**	**100**

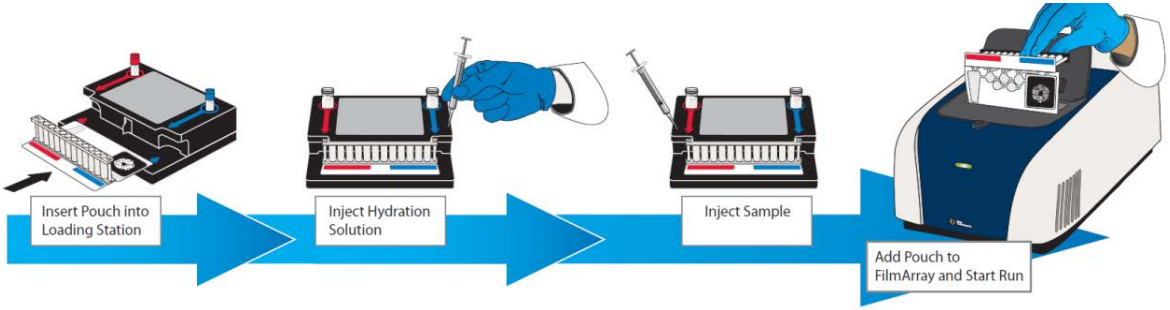
BioFire FilmArray

- Preparation of the pouch
- Add pouch to FilmArray station (overall, **2 min**)
- Run time of about **1 h**



Multiplex PCR
(RT-PCR for RNA target)

Multiplex real-time PCR
and Melting analysis



FilmArray® BCID Panel targets:

~100 Euro

Gram+ Bacteria	Gram– Bacteria
Enterococcus *Listeria monocytogenes* *Staphylococcus* *Staphylococcus aureus* *Streptococcus* *Streptococcus agalactiae* *Streptococcus pyogenes* *Streptococcus pneumoniae*	*Acinetobacter baumanniiHaemophilus influenzae* *Neisseria meningitidis* *Pseudomonas aeruginosa* *Enterobacteriaceae* *Enterobacter cloacae complex* *Escherichia coli* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Proteus* *Serratia marcescens*
Yeast	**Antibiotic Resistance**
Candida albicans *Candida glabrata* *Candida krusei* *Candida parapsilosis* *Candida tropicalis*	mecA - methicillin resistance vanA/B - vancomycin resistance KPC - carbapenem resistance

Evaluation of the FilmArray Blood Culture Identification Panel: Results of a Multicenter Controlled Trial

Salimnia H.. *et al.*, J Clin Microbiol; 54:3, 2016

8 centers in USA
July 2012 - Feb 2014
2,207 BC samples
(processed within 8 h)

Species ID	Isolates detected[a]: BCID/comparator		No. of results: BCID/comparator					
	Clinical arm	Seeded arm	TP +/+	FP +/−	FN −/+	TN −/−	Sensitivity or PPA[b]: TP/(TP + FN) (%)	Specificity or NPA[b]: TN/(TN + FP) (%)
Gram-positive bacteria								
Enterococcus	102/101	29/29	127	4	3	2,073	127/130 (97.7)	2,073/2,077 (99.8)
L. monocytogenes	0/0	36/36	36	0	0	2,171	36/36 (100)	2,171/2,171 (100)
Staphylococcus	780/797	2/1	770	12	28	1,397	770/798 (96.5)	1,397/1,409 (99.1)
S. aureus	257/257	0/0	253	4	4	1,946	253/257 (98.4)	1,946/1,950 (99.8)
Streptococcus	140/141	63/62	198	5	5	1,999	198/203 (97.5)	1,999/2,004 (99.8)
S. agalactiae (group B)	18/18	18/18	36	0	0	2,171	36/36 (100)	2,171/2,171 (100)
S. pneumoniae	26/25	12/12	36	2	1	2,168	36/37 (97.3)	2,168/2,170 (99.9)
S. pyogenes (group A)	8/7	31/31	38	1	0	2,168	38/38 (100)	2,168/2,169 (99.9)
Total	1,331/1,346	191/189	1,494	28	41	16,093	1,494/1,535 (97.3)	16,093/16,121 (99.8)
Gram-negative bacteria								
A. baumannii	16/14	40/37	51	5	0	2,151	51/51 (100)	2,151/2,156 (99.8)
Enterobacteriaceae	307/310	187/188	490	4	8	1,705	490/498 (98.4)	1,705/1,709 (99.8)
E. cloacae complex	24/22	17/17	38	3	1	2,165	38/39 (97.4)	2,165/2,168 (99.9)
E. coli	149/148	6/5	150	5	3	2,049	150/153 (98.0)	2,049/2,054 (99.8)
K. oxytoca	6/6	54/58	59	1	5	2,142	59/64 (92.2)[c]	2,142/2,143 (99.9)
K. pneumoniae	74/71	37/34	102	9	3	2,093	102/105 (97.1)	2,093/2,102 (99.6)
Proteus	22/22	17/17	39	0	0	2,168	39/39 (100)	2,168/2,168 (100)
S. marcescens	22/22	55/55	76	1	1	2,129	76/77 (98.7)	2,129/2,130 (99.9)
H. influenzae	8/8	35/35	43	0	0	2,164	43/43 (100)	2,164/2,164 (100)
N. meningitidis	1/1	35/35	36	0	0	2,171	36/36 (100)	2,171/2,171 (100)
P. aeruginosa	52/52	0/0	51	1	1	2,154	51/52 (98.1)	2,154/2,155 (99.9)
Total	681/676	483/481	1135	29	22	23,091	1,135/1,157 (98.1)	23,091/23,120 (99.9)

Drug-Resistant Organisms	Isolates detected: BCID/comparator		No. of results: BCID/comparator					
	Clinical arm	Seeded arm	TP +/+	FP +/−	FN −/+	TN −/−	Sensitivity or PPA[a]: TP/(TP + FN) (%)	Specificity or NPA[a]: TN/(TN + FP) (%)
mecA in association with:								
All *Staphylococcus* isolates detected[b]	491/494	2/2	488	5	8	281	488/496 (98.4)	281/286 (98.3)
Staphylococcus and *S. aureus* isolates detected	137/139	0/0	137	0	2	118	137/139 (98.6)	118/118 (100)
vanA/B in association with *Enterococcus* isolates detected	36/36	28/28	64	0	0	67	64/64 (100)[c]	67/67 (100)
bla_{KPC} in association with *Enterobacteriaceae* and/or *A. baumannii* and/or *P. aeruginosa* isolates detected	6/6[d]	33/33	39	0	0	558	39/39 (100)[e]	558/558 (100)

Only KPC!

Emergence of *Klebsiella pneumoniae* co-producing NDM-1, OXA-48, CTX-M-15, CMY-16, QnrA and ArmA in Switzerland

IJAA, 2014

Salome N. Seiffert[a,b,c], Jonas Marschall[d], Vincent Perreten[b], Alessandra Carattoli[a,b,e], Hansjakob Furrer[d], Andrea Endimiani[a,*]

Antibiotic MIC, in µg/mL (interpretation)[a]	
Piperacillin/tazobactam	≥128 (R)
Ticarcillin/clavulanic acid	≥256 (R)
Cefoxitin	≥128 (NA)
Cefpodoxime	≥64 (R)
Ceftriaxone	≥256 (R)
Cefotaxime	≥128 (R)
Cefotaxime/clavulanic acid	≥128 (NA)
Ceftazidime	≥256 (R)
Ceftazidime/clavulanic acid	≥256 (NA)
Cefepime	≥32 (R)
Aztreonam	≥32 (R)
Imipenem	4 (R)
Meropenem	≥16 (R)
Ertapenem	≥8 (R)
Doripenem	≥4 (R)
Gentamicin	≥32 (R)
Tobramycin	≥16 (R)
Amikacin	≥64 (R)
Ciprofloxacin	≥4 (R)
Levofloxacin	≥16 (R)
Trimethoprim/sulfamethoxazole	≥8 (R)
Colistin	≤0.125 (S)
Polymyxin B	≤0.125 (NA)
Doxycycline	16 (NA)
Minocycline	8 (NA)
Tigecycline	0.5 (S)
Fosfomycin[b]	2 (S)
Resistance genes (genetic background or characteristics)	bla_{SHV-1} (NS) bla_{TEM-1} (NS) $bla_{CTX-M-15}$ (ISEcp1) bla_{CMY-16} (ISEcp1) bla_{OXA-48} (Tn1999.2) bla_{NDM-1} (ISAb125) OmpK35 (stop codon) armA (NS) GyrA (Ser83Tyr; Asp87Asn) ParC (Ser80Ile) qnrA $bla_{OXA-7-like}$, arr-1, dfrA12, dfrA14, sul1, sul2, cmlA1, floR, aadA1, tet(A), IntI1[c]
Plasmid incompatibility (Inc) group	A/C, R, L/M
MLST	ST101

IncL/M
OXA-48
Carbapenems

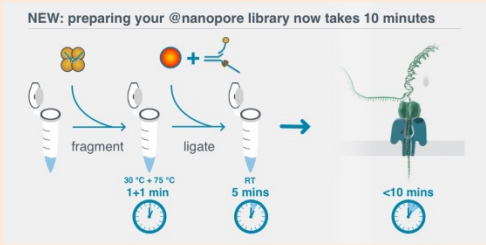
IncR
CTX-M-15
Penicillins
Cephalosporins
Monobactams
Bactrim

IncA/C
NDM-1
CMY-16
Penicillins
Cephalosporins
Monobactams
Carbapenems
Bactrim
Tetracyclines
Chloramphenicol
Aminoglycosides
Quinolones

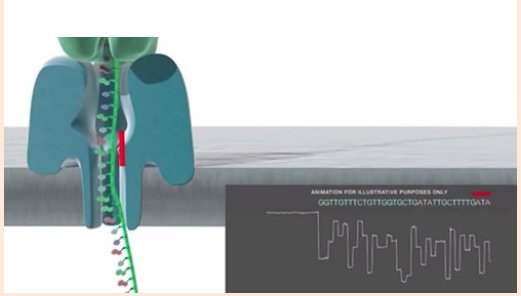
2-3 weeks work for the PhD student

Oxford NANOPORE Technologies

Real-time long read whole genome sequencing
(Point of care, POC)



150-600
Euro



1-2 hrs enough reads/coverage
To reports all resistance genes

Conclusions

Carbapenem-resistant strains have high prevalence and still increase

- Most of them are carbapenemase producers
- KPC, NDM, and OXA-48 are the most frequent enzymes
- The genetic background and special clones support their spread

Standard phenotypic tests do not detect all carbapenemase producers

- The clinical breakpoints do not identify 15-20% of strains
- Screening tests may improve detection (if implemented)
    - They are cheap, but time consuming (1-3 days)

Non-molecular rapid tests

- They are cheap and relatively fast, but provide only a generic detection

Molecular rapid tests

- Most identify only one or few carbapenemase genes
- Most do not identify species and other important genes (ideal system!)
- Only some are validated for clinical samples
- They are expensive

THANK YOU!

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